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INVESTIGATION OF CRIMEAN-CONGO HEMORRHAGIC FEVER
AND HEMORRHAGIC FEVER WITH RENAL SYNDROME IN GREECE

MIDTERM REPORT

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13. ABSTRACT (Maximum 200 words) <p>Techniques used in this investigation such as IFA and ELISA tests, and Hantaan and CCHF virus isolation from several pathological specimens were described in previous reports. Further on, data concerning the follow up of the renal function of patients with HFRS, and data concerning the detection of Hantavirus antibodies among hypertensive individuals and patients with glomerular disease of unknown origin are collected and they will be statistically analyzed in the future.</p>				
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Hemorrhagic fever with renal syndrome.

A1. Serological diagnosis of HFRS cases.

One hundred and sixty one (161) blood samples (single or paired) were taken from HFRS suspected patients and examined by IFA and μ -capture Elisa assays for antibodies to Hantavirus. In 11 out of the 161 patients the disease was due to infection with Hantaan virus. These 11 patients had rise titres of IgG and IgM antibodies to Hantaan virus. High titres of IgM and IgG specific antibodies were found and the titres were corresponding to the day of the disease the sample was taken. Four out of the eleven patients developed hemorrhagic phenomena. Five of patients were haemodialysed and all the patients survived. Cases were sporadic in different localities of Northern Greece. Hematological, chemistry, and renal function tests have been studied and all the data are kept for statistical analysis.

A2. Attempts for virus isolation from patients.

Blood and urine samples were taken from 5 of the 11 patients for virus isolation. Whole blood as well as urine samples were inoculated into 25-cm² flasks containing Vero E-6 cells and the attempt for virus isolation is on process. Lymphocytes for virus isolation were taken from the whole blood of the 6 patients, activated with PHA and IL2 was added. Patient's

passages (6) lymphocytes obtained from two patients showed fluorescence by IFA test using specific human and rat sera. Supernatant culture fluids as well as lymphocytes are kept at liquid Nitrogen for further investigation.

A3. Attempts for virus isolation from small mammals.

Eight-six small mammals were live-trapped in fields and forests surrounding 3 villages where previously HFRS cases were diagnosed serologically. Captured mammals were transferred alive to the University laboratory in Thessaloniki for virus isolation. Whole blood was obtained with capillary tube Pasteur pipette with retroorbital sinus rupture. The serum was examined for antibody to Hantaan virus by IFA assays. Lungs, kidneys and spleen were aseptically removed. Kidneys and spleens were stored at -70 C whereas the lung tissues from seropositive rodents were dissociated with a mechanical blender and were inoculated into 25-cm² plastic flasks containing Vero E-6 cells. After four passages (15 days each) all the samples were negative when examined by IFA test using specific human and rat sera. Additionally, when monoclonal antibody were used, the results were also negative.

A4. Human serosurvey for antibody to Hantavirus.

One thousand two hundred and forty-nine blood samples were collected from the residents of villages where HFRS cases were previously serologically diagnosed and from other localities. Thus blood samples were collected from residents of Florina county (112 samples), Kilikis county (218 samples), Komotini county (270 samples), sera county (199 samples), Evros county (96 samples) and from Samos (260 samples) islands. Sera were examined by IFA test and the overall antibody prevalence rate was 3,1% with a range from 0,8% (Thasos island) to 3,5% (Florina county).

A5. Serosurvey for antibody to Hantaan virus among hypertensive individuals.

Four hundred and seven (407) human sera were taken from hypertensive individuals whose hypertension was of unknown origin. The blood samples were provided by several "Hypertensive Units" of different hospitals. Hypertensive individuals were identified by age, sex, location of residence and history of renal failure. The overall antibody prevalence rate was 2,8% with a range from 0 to 6% depending on the locality where the individuals are residents. However, these data need further statistical analysis.

A6. Serosurveys for antibody to Hantavirus among patients with glomerular disease of unknown origin.

Two hundred and seventy-five human sera were obtained from patients with glomerular disease of unknown origin. The blood samples were provided by Renal Units of AHEPA, Agia Sophia and Ioannina University Hospitals. The overall antibody prevalence rate was 4,6% with a range from 1,1 to 6,3% and the maximum percentage of positivity occurred in areas where clinical disease was diagnosed.

A7. Follow-up of the renal function in previously diagnosed HFRS patients.

Hospital appointments were given to 43 previously hospitalized HFRS patients. Only 21 out of the 43 previously hospitalized patients were accepted the appointment and visited the hospital. Blood samples as well as urine samples were taken for renal function tests, and hematology and chemistry blood tests. Six of the 21 previous hospitalized patients were discharged 5 years ago, 4 three years ago, 6 two years ago and 5 one year ago. Comparing the renal function of the patients on the time of their discharge with the renal function of their appointment we found out that in 17 patients, the filtration rate (GFR) was within normal limits and in 4 patients a degree of GFR reduction was remained. Five patients had renal tubular acidosis and urine pH <5.8. 2 of the patients with normal GFR had reduced concentration

ability. All data concerning renal function tests, hematology and chemistry tests will be statistical analyzed previously hospitalized HFRS patient will participate in this investigation.

B. Crimean-Congo hemorrhagic fever

B1. Human surveys for antibody to CCHF virus.

One thousand and forty-nine blood samples (the same samples which were used for Hantavirus serosurvey) were examined by IFA and ELISA test for antibodies to CCHF virus. The overall antibody prevalence rate by IFA and ELISA was 1,1 and 2% respectively with a range by IFA test from 0% (Thasos island to 3,1% (Kastoria county).

B2. Attempts to diagnose human C-CHF cases.

Three hundred and seventy-three blood samples were obtained from patients with clinical picture resembling C-CHF 35 patients, with pyrexia of unknown origin and elevated liver enzymes 184, influenza-like disease 155. Patient's sera were examined by IFA and ELISA assays for IgG and IgM antibodies to C-CHF virus. None of the examined patients was found to be infected with C-CHF virus.

B3. Animal serosurvey for antibodies to C-CHF.

Animal serosurvey for antibodies to CCHF virus. Nine hundred and fifty-nine blood samples were collected from sheep (452 samples) and goats (507 samples). Animals were pastured in Grammos (264 sheep and goats), in Drama county (116 sheep and goat), Samos island (358 sheep and goats). The overall antibody prevalence by IFA and ELISA was 2,4 and 3,4% respectively with a range from to 6,1% (Grammos). Goats are more often infected, ratio goat to sheep 3:2 respectively.

B4. Attempts for C-CHF virus isolation from ticks.

Two thousand two hundred and seventy-seven ticks were collected from goats and sheep pastureied in Drama county (316 ticks) in Grammos (271 ticks) and Krete island (1200 ticks). Half of the ticks were pooled and the supernatant of each pool was inoculated into 25 cm² plastic flasks containing Vero E-6 cells for C-CHF virus isolation. After six days of incubation at 37° C, spot-slides were prepared from each flask for detection CCHF antigen in the cells by IFA test. Thus far, isolation of CCHF virus from ticks was not succeeded.

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